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13. ABSTRACT (Maximum 200 words) The serpulid polychaete, <u>Hydroides elegans</u> , is a prominent fouling organism in tropical marine waters. Fouling is first caused by the formation of an unmineralized primary tube and thread by settling larvae. This is replaced by a mineralized secondary attachment tube during metamorphosis. Biochemical analysis of the primary and secondary tubes suggests a composition rich in acidic amino acids and glycine. Dopa is transiently present in new growth. The latter finding, particularly, may indicate a functional similarity to the adhesive proteins of mussels, ascidians, and sabellariids.			
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FINAL REPORT

GRANT #: N00014-95-1-1015

PRINCIPAL INVESTIGATOR: Herbert Waite

INSTITUTION: University of Delaware; Subcontractor: University of Hawaii
(submitting a separate report).

GRANT TITLE: The biochemistry of primary attachment in the serpulid
larvae *Hydroides elegans*.

AWARD PERIOD: 1 July 1995-31 Dec 1998

OBJECTIVE: To identify the proteins of primary attachment in the fouling
polychaete *Hydroides elegans*.

APPROACH: There were several approaches: 1. Originally our approach was
to extract and purify proteins from the primary tube of *H. elegans*
(provided by Dr. M. Hadfield at Hawaii);. 2. Later efforts shifted to
isolation of adhesion-related redox-active molecules from presettlement-
competent larvae. 3. Finally, we tried to characterize proteins/peptides
from juvenile tubes with the hope of tracing their expression back to
primary attachment. The general approach was to release peptides from
the insoluble attachment structure by proteolytic treatment; to purify
and sequence the liberated peptides, and then, with degenerate
oligonucleotide primers based on peptide sequences, to use RT-PCR
strategies to derive first partial and then complete cDNA sequences. The
protein/peptide chemistry was done at Delaware: the molecular work was
to be done at Hawaii.

ACCOMPLISHMENTS: Using a micromanipulator, we manually dissected out
enough primary tubes (~20) for an amino acid analysis which suggests a
aspartic acid/glutamic acid/glycine rich protein. The same three amino
acids plus serine dominated the composition of primary threads. Our
efforts to characterize individual proteins or peptides from the primary
tubes were limited for two reasons: inadequate tube material (<50 μ g) and
inability to separate larvae from the primary tubes. Since the primary
tubes and associated threads were redox-active with nitroblue
tetrazolium (NBT - an indicator for quinones), we looked for expression
of redox-active molecules in larvae at 2, 3 and 4 days of development
post fertilization. Attachment competency usually begins at 3-4 days.
Expression of redox-active components was highest at 2-3 days. Proteins
were extracted from 2-day old larvae using 5% acetic acid, separated by
C-18 HPLC and assayed by reaction with NBT. A peak eluting at 30 min
reacted strongly with NBT. Sequence and mass were not obtainable due to
heterogeneity and inadequate material. The final strategy to isolate
proteins/peptides from demineralized juvenile/adult tubes did offer
slightly more working material (100 mg), however, the yield of soluble
peptides was always less than 0.1% of the starting material. We tried
several proteolytic treatments: pepsin, chymotrypsin, lys C-
endoproteinase, trypsin and cyanogen bromide which consistently
liberated 24, 5, 6, 8 and 1 peak(s), respectively, by C-18 HPLC. Most

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were Glu, Gly and Asp rich. Only two gave clean Edman sequence: the CNBr treatment produced Tyr. This suggests that many of the proteins had a C-terminal sequence Met-Tyr. Lys-C endopeptidase produced many peaks by HPLC but one was a clean peptide: DAEDDDDD. Trypsinization of dissected new growth increments from adult tubes at Hawaii in Feb 1997 gave a strong indication of DOPA: Highest levels in C-18 HPLC peaks approached 1-2 mol %.

CONCLUSIONS: The adhesive proteins present in the primary and juvenile tubes of post-larval Hydroides elegans are probably different gene products but have significant similarities: Both contain quinone-like redox activity and both have amino acid compositions rich in aspartic/glutamic acid and glycine. Peptides released by proteolysis from the juvenile and adult tubes contain 3,4-dihydroxyphenylalanine (DOPA). Adhesive protein constituents of juvenile tubes are completely insoluble. Future efforts would do well to concentrate on isolating and characterizing soluble DOPA-containing precursor proteins from adult worms, then use immunochemical and sequence resources to determine life history-specific expression and distribution patterns. We recommend in the strongest possible terms that future investigations of Hydroides attachment biochemistry be conducted exclusively at the sites of worm collection. This is based on our experience with the rapidity of cement maturation and the chemical instability of proteins associated with attachment.

SIGNIFICANCE: Like mussels, sabellariids, tunicates and gorgonian corals, adhesion in serpulids may be based on DOPA-proteins. Although a significant base of sequence information has yet to be compiled, proteins from the tubes of Hydroides appear to be rather acidic.

PATENT INFORMATION: None.

AWARD INFORMATION: Waite was awarded the Maxwell & Mildred Harrington Professorship in July, 1998.

PUBLICATION AND ABSTRACTS:

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